TYPE I COLLAGEN AS A MARKER OF BONE METABOLISM IN SICKLE CELL HEMOGLOBINOPATHIES

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Avascular necrosis of the skeletal system is a complication of sickle cell hemoglobinopathies. Type I collagen, which is synthesized by osteoblasts, comprises about 90% of the total organic matrix of bone. This preliminary study of skeletal changes in sickle cell hemoglobinopathies measured type I collagen formation and degradation using specific radioimmunoassays to determine the plasma concentrations of carboxyterminal propeptide of type I procollagen (PICP) and carboxyterminal pyridinoline cross-linked telopeptide of type I collagen (ICTP), respectively. Plasma ICTP concentrations were increased significantly in sickle cell patients with no clinical or radiologic symptoms of bone complications than in controls. Mean plasma ICTP concentration was moderately high in all patients. Plasma PICP levels did not differ significantly between patients and controls. This preliminary results indicate that measurements of plasma ICTP might be helpful in predicting bone changes in sickle cell hemoglobinopathies and also useful in the early detection of skeletal complications of the disease. (*J Natl Med Assoc.* 1998;90:41-45.)

Key words: bone ◆ sickle cell hemoglobinopathies ◆ type I collagen

Sickle cell hemoglobinopathies are probably the most common cause of avascular necrosis of the bone.[1-4] The two major factors that contribute to avascular necrosis are chronic hemolytic anemia, which often causes bone marrow hyperplasia, and sickling of the erythrocytes, which causes bone infarcts.[5] Most of the bone infarcts in these patients are found in areas supplied by end arteries, especially the heads of the long bones such as the femur and humerus,[5] although other bones also are affected. More than 30% of sickle cell patients end up with complications of avascular necrosis of the bone.[1-5]

The disappointing results of therapy of avascular necrosis after bony collapse have led to the suggestion by many workers that future efforts should be directed to the early detection or diagnosis of this bone disease before the onset of the skeletal or structural failure in these patients.[5] While bone scan with technetium ^{99m}Tc and magnetic resonance imaging can detect disease, these tests are expensive and difficult to use on a regular basis.[5-8]

Type I collagen accounts for 90% of the bone organic matrix and is synthesized by the osteoblasts.[9-11] The synthesis of type I collagen can be followed by measuring the plasma or serum levels of carboxyterminal propeptide of type I procol-

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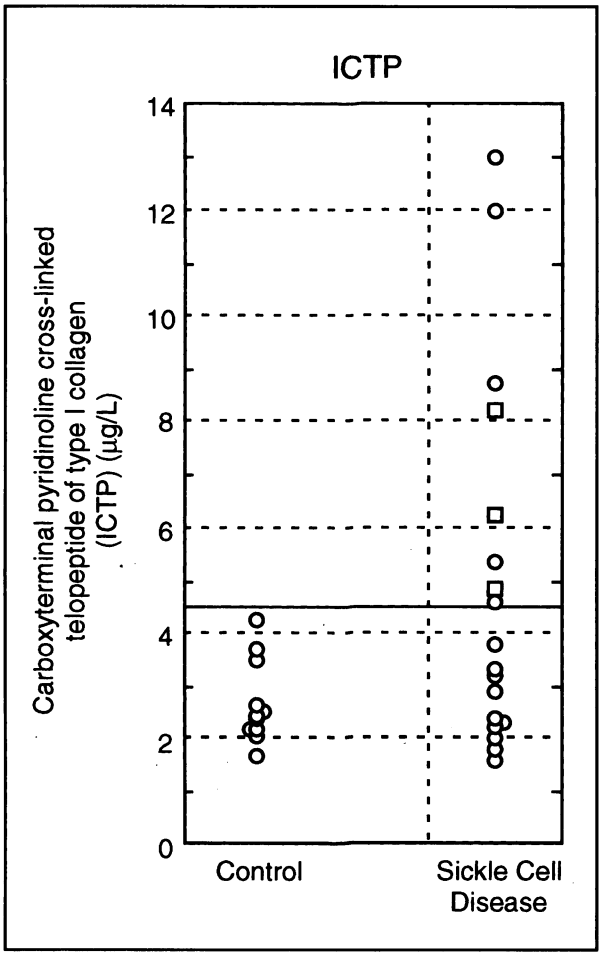

Figure 1.
Carboxyterminal pyridinoline cross-linked telopeptide of type I collagen (ICTP) in controls and patients with sickle cell hemoglobinopathies. The horizontal line indicates the normal mean+2 standard deviations of the controls. (□=sickle cell patients with clinically identified bone complications.)

lagen (PICP).[9,10] The PICP molecule is too large to be excreted through the kidneys. Instead, it is cleared through the liver endothelial cells,[9,12] which has to be considered when evaluating the values in patients with liver disease.[9]

Degradation of type I collagen also can be studied by using a new radioimmunoassay that measures the serum or plasma concentration of carboxyterminal pyridinoline cross-linked telopeptide of type I collagen (ICTP).[10,11] In the collagen fibers, collagen molecules still undergo chemical modification. Intermolecular cross-links are formed between the terminal, nonhelical telopeptide parts of one type I collagen molecule, and the helical region of another.[11] Thus, the carboxyterminal telopeptide of type I collagen is formed extracellularly in only matured collagen molecule.[13] During the degradation of bone tissue, these cross-linking peptide regions are released into the bloodstream.[10-13] ICTP is not further metabolized or degraded but is released as an intact fragment and cleared from circulation through the kidneys.[11-13] Blood levels of ICTP therefore correlate well with rates of bone resorption measured histomorphometrically.[12,13]

This preliminary study was undertaken to investigate whether plasma metabolites of type I collagen metabolism can be used as a noninvasive and early diagnostic marker of possible alterations in bone or skeletal changes in patients with sickle cell hemoglobinopathies.

MATERIALS AND METHODS

Study Population

Eighteen patients (7 males and 11 females) with sickle cell hemoglobinopathies were studied. Mean age (standard deviation [SD]) of the patients was 25.58 (8.12) years (range: 18 to 43 years). A reference control group of 10 apparently healthy subjects (4 males and 6 females) also was included in the investigation. The mean age (SD) of the control subjects was 25.70 (8.45) years (range: 18 to 44 years). Subjects had no history of fractures nor any nutritional, metabolic, liver, renal, or bone disease, and none of the females were on contraceptives prior to obtaining blood samples. All of the control subjects had hemoglobin AA.

The diagnosis of sickle cell hemoglobinopathies was based on the hemoglobin pattern on cellulose acetate electrophoresis. Family studies were consistent where available. The genotypes of the patients were divided as follows:

- SS-sickle cell anemia (14 patients),
- Sβ-sickle cell thalassemia (2 patients), and
- SC-sickle cell hemoglobin C disease (2 patients).

Three patients were identified clinically and radiologically to have bone complications due to sickle cell hemoglobinopathies. All other patients were asymptomatic for skeletal complications at the time blood samples were taken for the assays. The clinical history showed that none of the patients had liver, renal, or inflammatory disease, and none showed symptoms of these complications during the time blood samples were taken.

Blood Samples

Plasma samples for the measurement of PICP

and ICTP were random preserved samples remaining after routine tests for patients confirmed to have sickle cell hemoglobinopathies. Samples were stored at −20 °C until analyzed. Assays were carried out within 1 to 2 weeks of storage.

ICTP and PICP Assays

Plasma levels of ICTP were determined in duplicate 100-mL samples using a radioimmunoassay kit from Incstar Corp (Stillwater, Minnesota). The intra-assay coefficients of variation (CV) were constantly around 6.1%, and the inter-assay CV was 7.5%. Plasma PICP also was measured using a radioimmunoassay kit from Incstar Corp (Stillwater, Minnesota). The intra- and inter-assay CV were 3.5% and 5.5%, respectively.

Data Analysis

Results are expressed as means±SD and compared as applicable using ANOVA and Student's *t*-test. Correlations were determined using linear regression analysis.

RESULTS

The control values for ICTP and PICP were determined from the plasma of 10 healthy subjects assayed together with the plasma of the patients. Mean (±SD) plasma ICTP and PICP concentrations in the 10 controls were 2.7±0.9 mg/L and 159±74 µg/L, respectively. Thus, values >4.4 mg/L (control mean+2 SDs) were considered abnormal for ICTP (Figure 1), and values >307 µg/L were considered abnormal for PICP (Figure 2).

Mean plasma ICTP (4.9±3.5 mg/L) was moderately elevated ($P<.07$) in all patients with sickle cell hemoglobinopathies compared with controls. Mean plasma PICP concentrations did not differ significantly ($P=.18$) in patients (216±116 µg/L) compared with controls.

Plasma ICTP was above the reference range in 8 (44 %) of 18 patients (Figure 1), and PICP was elevated above the reference range in 5 (28 %) of 18 patients (Figure 2). Three of the eight patients with pathologically increased ICTP levels had clinical and radiological bone complications (Figure 1). The mean plasma level in the remaining five patients with no clinical or radiological skeletal complications differed significantly ($P<.008$) from the control. In addition to increased plasma PICP levels, three out of the five patients also had elevated ICTP levels (Figure 2). One of these three patients had clinically identified bone complications (Figure 2). There was no correlation between these two markers.

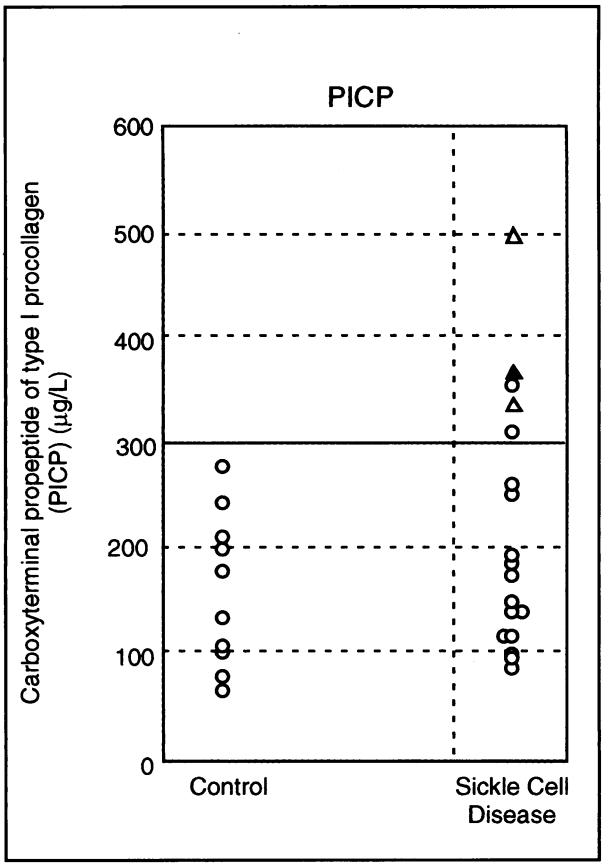

Figure 2.
Carboxyterminal propeptide of type I procollagen (PICP) in controls and patients with sickle cell hemoglobinopathies. The horizontal line indicates the normal mean+2 standard deviations of the control. (Δ=those patients who, in addition to increased PICP concentration, also had increased ICTP concentration and ▲=a sickle cell patient with clinically identified bone complication who had an increased ICTP level in addition to an elevated plasma PICP level.)

DISCUSSION

These preliminary findings in the assays of plasma biochemical markers of type I collagen metabolism in patients with sickle cell hemoglobinopathies correlated with the clinical and radiological findings of avascular necrosis in some patients with this disease.[1-6] In this study, type I collagen metabolism was determined by measuring biochemical markers of type I collagen formation and degradation.[9-11] The advantage of determining PICP and ICTP lies in their high specificity for type I collagen,[12-15] which comprises

90% of the organic matrix of the skeletal system or bone.[9-11,12-14,16,17] The radioimmunoassays are easy to perform because of their reproducibility, and both measurements correlate well with bone mass as confirmed by histomorphometric studies.[9-11,12,16]

It has been shown immunohistochemically that types I and III collagen have a similar distribution within the hemopoietic compartment along the bone marrow sinusoids and in fat spaces.[18] Type III procollagen, which has not completely lost its aminoterminal propeptide, also was demonstrated immunohistochemically as a genuine component of the extracellular matrix fibers in human bone marrow.[19] Thus, measurements of serum aminoterminal propeptide of type II procollagen (PIIINP) have been suggested as a noninvasive marker of bone marrow fibrosis or myelofibrosis.[20,21] More recent studies using the serum PIIINP assay in children undergoing bone marrow transplantation have revealed positive correlation with the development of veno-occlusive liver disease in these patients.[22,23] The increases in serum PIIINP in veno-occlusive disease reflect the formation and intrahepatic accumulation of type III collagen.[22,23] Previous study of serum PIIINP in sickle cell hemoglobinopathies demonstrated increased levels and suggested that it may be due to diffuse fibrogenesis of the liver and other organs.[24]

Carboxyterminal pyridinoline cross-linked telopeptide of type I collagen exceeded the upper limit of the reference interval (normal mean+2 SDs) in 44% (8/18) of total patients studied and was elevated in all 3 of the patients who had clinical and radiologically identified skeletal complications. It also was significantly increased in a group of sickle cell patients with asymptomatic or no clinically identified bone complications. It remains to be determined if these patients are at increased risk of skeletal disease. Surprisingly, PICP was elevated in only five patients, ie, 28% (5/18) of the total patients studied. Three of these five patients with high levels of PICP also had elevated levels of ICTP, and one of these three patients had clinically identified bone complications.

CONCLUSION

Measurements of PICP and ICTP in plasma may give information on early skeletal changes in sickle cell hemoglobinopathies. These assays may allow early therapeutic intervention, thus preventing further bone destruction or loss and fracture. Serial measurements of serum PICP, ICTP, PIIINP, urinary aminoterminal telopeptide of type I collagen, and serum osteocalcin including bone alkaline phosphatase need to be performed to determine variations in association with bone infarcts, fibrotic changes in bone marrow, or episodes of aseptic necrosis.

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